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RESEARCH ARTICLE

SHELF-LIFE EXTENSION OF GERMINATED GREEN GRAM STUFFED PAROTHAS.

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Abstract

Stuffed parothas are perishable in nature and get spoiled within 24 hrs of their preparation. Armed Forces required food products having a longer shelf life with high nutritive value. Germination results an increase in nutritive value in terms of protein, fibre and minerals. Hence efforts were being made to develop shelf stable stuffed parothas using germinated green gram and permitted level of antimycotic agent with inpack heat treatment. Stuffed parothas were subjected to heat treatment at 90^{o}C for 2 hrs and stored at ambient temperature conditions (14-34^{0}C). Shelf-life of stuffed parothas was evaluated chemically, sensorily and microbiologically to assess their shelf-life. After 9 months of storage, peroxide value (PV), thiobarbituric acid value (TBA), free fatty acid value (FFA) and browning index increases significantly ($p \leq 0.05$) from 5.12 to 19.24 meqO2/Kg fat, 0.087 to 0.142 mg MA/Kg sample, 0.75 to 1.69 % oleic acid and 0.082 to 0.112 respectively. During storage, there was no significant decrease in sorbic acid content was observed. Negative correlation was observed between chemical changes and overall acceptability during storage. During storage, textural and colour values had an impact on sensory scores which decreases significantly ($p \leq 0.05$) from 8.5 to 7.5 on a nine point hedonic scale. Microbiologically stuffed parothas remained stable during entire period of storage. Highly acceptable and nutritious germinated green gram stuffed parothas with longer shelf-life can be prepared using antimycotic agent and inpack heat treatment. The product remain safe chemically and microbiologically upto 9 months of storage at ambient temperature conditions.

Introduction:-

Wheat is world's most important cereal crop in terms of production. In India more than 80% of wheat produced is consumed in the form of traditional products such as chapati (an Indian unleavened bread) and other bakery products and are relished by all the segments of population (Khan, 2012). Cereals are important sources of energy and protein in human diets. Although carbohydrates are their main dietary contribution, they provide protein and smaller amount of lipids, antioxidants, dietary fibre and vitamins. It is commonly known that the main nutritional drawback in cereals, particularly wheat (*Triticum aestivum*) is their low protein content and the limited biological quality of their protein (highly deficient in lysine and tryptophan) compared to animal protein. No one legume or cereal can provide adequate amounts of all nutrients to meet the nutritional requirements. Pulses combined with

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cereals offer the most practical way of solving the problem of protein malnutrition in countries like India (Sharma and Chopra, 2015).

Green gram (*Vigna radiate*) belongs to the family Leguminoseae and is an important food legume and is widely cultivated in tropical and subtropical conditions and is believed to be native crop of India and Central Asia (Puranik et al., 2011; Chandrasekhar and Ghosh, 2002). Green gram contains higher proportion of lysine and can be processed and consumed as cooked whole beans or splits, sprouts immature seeds and flour and are used in various recipes. Among other pulses, green gram is a good source of vitamins, minerals, enzymes, complex carbohydrates etc (Puranik et al., 2011; Jood et al., 1998). Green gram seeds are preferred for feeding babies as it is known for its easy digestibility, low flatulence potential(Doughty and Walker, 1982).

Although legumes are rich in complex carbohydrates (dietary fibres) and sources of minerals and vitamins but their efficient utilisation is affected by the presence of anti-nutritional factors viz tannins, phytins and trypsin inhibitors. Sprouting or germination not only improves the protein / amino acid digestibility by decreasing anti-nutritional factors but also, activates enzymes present in the grain thus converting starch to fermentable sugars, partial hydrolysis of proteins and other macromolecules (Bibi et al.1997; Potter and Hotchkiss, 1995). They also reported presence of higher levels of nutrients and lower levels of anti-nutritional factors in sprouts compared to non-germinated seeds. Malnutrition is a major problem in underdeveloped countries. Priyanka et al (2016); Priyanka and Anita (2015) developed ready-to-eat supplementary foods based on germinated cereal-pulses mix, potato flour and green leafy vegetables to overcome malnutrition in children and recommended the use of products for supplementary feeding programmes. Dipika et al. (2013) developed multi nutrient mixes using combination of cereal, pulses, soy protein isolate, sprouted green gram etc and reported that sprouting / malting had marked effect on physical properties of mixes and also found to be a useful processing in improving the quality and functionality of the mixes.

Parotha is a flat non leavened Indian bread which is a layer of cooked dough (Khan et al., 2012). Stuffed parothas are made using different filler ingredients viz potato, paneer, radish etc based on the consumer choice and are relished by all segments of population. Normally chapaties or roti which is also Indian unleavened bread is consumed along with adjuncts like gravy, pickle etc. Stuffed parothas can be consumed without any adjuncts just by warming on a hot plate or as such. Stuffed parothas are perishable and get spoiled within 24-48 hrs or preparation. Earlier Khan et al (2012) studied the effect of thermal processing on the development of potato stuffed parothas and paneer stuffed parothas and found that the shelf-life of these parothas can be enhanced to one year under ambient temperature conditions. The shelf-life of germinated green gram pickle using class one preservative was evaluated by Puranik et al (2015) and concluded that germinated green gram pickle was found to be nutritious in terms of high protein and fibre. Sharma and Chopra (2015) developed biscuits by partially replacing refined wheat flour with germinated green gram flour and reported an increase in protein, iron and calcium of biscuits prepared using combination of 40% malted flour with improved nutrient content. Earlier many attempts have been made to formulate malted weaning foods using ragi and green gram (Malleshi et al., 1986). Though literature is available on the development of various types of stuffed parothas using different ingredients and also use of green gram and germinated green gram in the development of products, but information is scanty on the development of stuffed parothas using germinated green gram and also to enhance its shelf-life using antimycotic agent. So efforts were made to standardise recipe for the development of nutritious germinated green gram stuffed parotha and to enhance its shelf-life using antimycotic agent along with inpack heat treatment.

Materials and Methods:-

Ingredient used for the preparation of germinated green gram was procured from local market. Sorbic acid and citric acid were procured from M/s Loba chemicals, New Delhi and M/s Reachem Lab Chemicals Pvt Ltd., Madras respectively. Packaging materials viz polypropyelene (75µ) and paper (45GSM)-aluminim foil (20µ)-polypropylene (37.5 µ) laminate pouches were procured from M/s India Foil Ltd., Calcutta.

Germination of green gram:-

Germination of green gram was carried out according to the method of Sharma and Chopra (2015). Cleaned green gram grains were soaked in water for 18 hrs at room temperature and allowed to germinate for 72 hrs. They were mixed regularly and watered when appeared dry. Germinated green gram grains were packed in suitable packaging material and kept at 4^{o}C for further use.

Preparation of Dough:-
Dough was prepared according to the method of khan et al (2014).

Preparation of germinated green gram Stuffing:-
Cooked and coarsely grounded germinated green gram (500g) was seasoned with known quantity of chilli powder (17.5g), cumin seed powder (7.5g), turmeric powder (7.5g), dhania powder (7.5g), ginger garlic paste (15g), garam masala (10g), cumin seeds (12.5g) hydrogenated fat (100g) etc till the temperature attains 90^{o}C to obtained desired moisture content and used for the preparation of germinated green gram stuffed parotha.

Use of Antimycotic Agent:-
For enhancement of shelf-life of stuffed parothas, combination of antimycotic agent sorbic acid (0.15%) and citric acid (0.075%) were used individually in dough as well as in stuffing in the form of emulsion.

Standardisation of stuffing and dough ratio for the preparation of stuffed parothas:-
Stuffing and dough ratio for the preparation of germinated green gram stuffed parothas was standardised by sensory quality evaluation. Seasoned stuffing was stuffed in dough rolled into a circular disc of 7″ diameter and either side was baked for 2–3 min at 210–220°C with the application of hydrogenated fat till golden brown colour is obtained.

Stuffed parothas prepared using different formulation of stuffing and dough were subjected to sensory evaluation by a panel of 15 semi trained judges by grading for taste and overall acceptability on a 9 point hedonic scale with 9 as excellent in all respects and 1 as unacceptable sample (Table 1). Based on sensory scores germinated green gram stuffing and dough ratio for the preparation of stuffed parothas was found to be 40:60.

Packing and Inpack Heat Treatment:-
Two numbers of stuffed parothas having butter paper lining in between were packed in Polypropylene (75µ, PP) pouches, sealed hermetically and inpack heat treated in a hot air oven at 90^{o}C for 2 hrs. Finally they were packed in paper (45 GSM)-aluminum foil (20 µ)-polyethylene (37.5 µ) laminate pouches (PFP) and stored at ambient room temperature conditions (14-34^{0}C) for further storage studies.

Chemical Analysis:-
Moisture, protein, fat and total ash were determined using standard methods (AOAC, 1990). Storage changes in stuffed parothas were evaluated through peroxide value (PV) and free fatty acids (FFA) as per the method of AOCS (1990), while thiobarbituric acid (TBA) value was determined as per the method of Tarledgis et al., 1960. The browning intensity was measured by shaking 5g sample with 100ml (70%) ethanol for 2h, filtering and measuring optical density at 420nm. Microbial profiles of the germinated green gram stuffed parothas were determined using the petri plate method for standard plate count (SPC) on plate count agar, coliform count on violet red bile agar and yeast and mold counts on potato dextrose agar as per the method of APHA (1992). The vitamin B_1& B_2 and vitamin C contents were determined using the flourimetric method (AOAC,1997). Carotenoid was estimated using hexane-acetone solvent mixture by titrometric method (Ranganna, 1986). Atomic absorption spectrophotometer (Vario 6, Analytika Jena., Germany) was used to determine the presence of mineral contents in stuffed parothas.

Texture profile Analysis:-
Texture profile analysis of stuffed parothas was performed using a Texture Analyser Plus (Model No.01/TALS/LXE/ UK; LLOYD Instruments, Hampshire.UK). The bite test on stuffed parothas was carried out using the volvodke bite set designed to imitate incisor teeth shearing through a food sample. The set comprises of upper and lower teeth which during the test penetrate into the sample twice to obtain the peak force and the texture profile at the pre-set speed and position. The parothas strips (2x15mm) were axially compressed to 90% of their original height, avoiding fracture, force–time deformation curve was derived with a 50.0kg load cell applied at a crosshead speed of 10.0mm/ min. Attributes were calculated as follows; hardness value as the peak force (N) of the first compression of the sample; cohesiveness as the area of work during the second compression divided by the area of work during the first compression (dimensionless); springiness, the distance (mm) that the sample recovers after the first compression; chewiness (N mm) as the product of the attributes, gumminess and springiness which in sensory terms corresponds to the energy required to chew the food product. Ten measurements per replication were taken for all the textural analysis.

Colour values:-
The colour values in terms of L, a and b for stuffed parothas were measured using a Hunter Colour Meter (Data Lab; Silvasa, Gujarat, India) with illuminant D65 and 100 observer. A higher L value indicated a brighter or whiter sample. Values of a and b indicated the red-green and yellow-blue chromaticity respectively.

Statistical Analysis:-
Experiments were performed using a 2-way factorial design consisting of storage time and attributes studied. All the experiments were performed in triplicate and Analysis of Variance calculated using Statistica Software Version 7.0 of Stat Soft Incorporation, Tulsa OK, USA (Snedcor and Cochran, 1968).

Results and Discussion:-

Table 2 refers to the proximate composition of stuffed parothas prepared using green gram before and after germination. There was a significant ($p \leq 0.05$) increase in protein content from 9.22 to 10.8%; ash 2.72 to 3.5%; crude fibre 3.28 to 4.40%; vitamin C 1.78 to 3.62mg $100g^{-1}$; B_1 0.04 to 0.06 mg $100g^{-1}$; B_2 0.05 to 0.08 mg$100g^{-1}$ and carotenoids 12.20 to 18.42µg g^{-1} while there was no significant changes in fat content, pH and water activity of stuffed parothas prepared by using germinated and ungerminated green gram (Table 2). Earlier workers also reported an increase in nutritive value after germination (Sharma and Chopra, 2015; Rumiyati et al., 2012 and Rafiya et al., 2016).

Synergistic effect of antimycotic agent and inpack heat treatment on the quality of stuffed parothas prepared using germinated green gram was evaluated by monitoring different physico-chemical, sensory, textural and microbiological attributes at a regular interval of time. The chemical changes in stored stuffed parothas at ambient temperature were monitored on the basis of changes in peroxide value (PV), free fatty acid value (FFA), thiobarbituric acid value (TBA) and browning index and the data has been presented in Table 3. After 9 months of storage, PV, FFA and TBA were found to be increased significantly from 5.12 to 19.24 meqO_2 Kg^{-1} fat; 0.75 to 1.69 % Oleic acid and 0.087 to 0.142 mg MA Kg^{-1} sample respectively. Khan et al (2014) reported an increase in PV, FFA and TBA contents in potato and paneer stuffed parothas during storage and attributed the increase due to the breakage of long fatty acid chain into individual fatty acid moieties and also increased lipid hydrolysis at elevated temperature. Although, during storage, there was a hydrolysis of lipids by the naturally occurring lipases, but during baking lipase activity was destroyed and therefore formation of free fatty acids in stored parothas must have resulted from the decomposition of hydroperoxide (Khan et al., 2014). Processed stuffed parothas contained only solid, hence there had been no dilution of TBA reacting substances resulting in the increase in TBA values during storage. During storage, there were no significant changes in moisture content in stuffed parothas stored at ambient temperature conditions. After 9 months of storage, moisture content in stuffed parothas was found to decrease from 38.44 to 37.52%. The slight decrease in moisture content during storage, may be due to the fact that packaging material based on aluminium foil was reported to act as a barrier against mass transfer, light and micro-organism and thus the moisture content of the products was almost retained (Khan et al., 2014 ; Ghosh, 1980). The changes in browning in stuffed parothas were measured as optical density of the alcoholic extract at 420 nm during storage and the results have been represented in Table 3. It was found that after 9 months of storage, browning was found to increase significantly from 0.082 to 0.112. During storage, there was a significant decrease in vitamins viz B_1 & B_2,Vitamin C and carotenoid contents (table 4). Earlier Padmashree et al (2012) reported losses in vitamins during storage in composite cereal bar[25].

Table 5 refers colour values in terms of Lightness (L), redness (a) and yellowness (b) of stuffed parothas during storage. On storage, there was a decrease in lightness (L) and yellowness (b) significantly ($p \leq 0.05$) with the increase in redness (a) indicating the samples becomes darker and the redness of the samples has been changed during storage. The changes may be due to the maillard reaction between the sugar and amino acids (Khan et al., 2012; Barnwal et al., 2013). The level of Polyphenol oxidase (PPO) activity as well as various phenolic compounds present in the dough also resulted in the rate of change of brightness (Baik et al., 1995).

The changes in textural attributes viz hardness, springiness and chewiness of stuffed parothas during storage is shown in Table 6. After 9 months of storage, there was an increase in hardness and chewiness from 3.90 to 9.65 N and 0.61 to 0.1.20 Nmm respectively with the decrease in springiness from 0.71 to 0.49 mm. The changes in textural attributes may be due to the fact that during baking of stuffed parothas amylase and amylopectin get random conformation as a result of gelatinisation and on storage and cooling, they reorient themselves into helical structure leading to crystallisation of starch molecules resulting in the hardness of stuffed parothas (Arya et al, 1984).

Mineral composition of stuffed parothas was evaluated and found that stuffed parothas had potassium 438; sodium 1108; calcium 59.6; iron 3.28; zinc 1.52 and magnesium 24.0 mg $100g^{-1}$. After 9 months of storage there were no significant changes in mineral composition of stuffed parothas.

Overall acceptability scores which is taken as mean of colour, aroma, taste and texture attributes on a 9 point hedonic scale at ambient temperature are given in Table 3, where 9 was given for excellent in all respects and 1 for highly disliked samples[29]. From the data, it was observed that there was a gradual decrease in OAA during storage. After 9 months of storage, OAA decreased from 8.5 to 7.5. The decrease in OAA scores may be attributed to the changes in colour values, textural attributes and other chemical parameters during storage.

The data on the microbiological profile of the stuffed parothas are shown in Table 7. The microbiological data showed that no SPC, coliform and yeast and mold were found in the stored parothas samples upto 9 months. Hence stuffed parothas remained stable microbiologically during the entire storage period.

Correlation Analysis:-

The chemical changes and overall acceptability scores were found to be negatively correlated ($r<-0.96$) during storage. The correlation between PV & OAA, FFA & OAA, TBA & OAA and BI & OAA were found to be -0.96, -0.98, -0.99, -0.99 respectively. The negative correlation indicated that with the increase in PV, FFA, TBA and BI, the overall acceptability of stuffed parothas during storage decreased. Significant correlation ($p\leq0.05$) was observed between the textural properties like hardness, springiness, stiffness and chewiness and overall acceptability scores, and it ranged from -0.99 to -0.97. Correlations between colour values obtained by using hunter colorimeter and their effect on sensory scores of stuffed parothas during storage were analyzed. It was found that lightness and yellowness were negative correlated ($r=-0.91$, $p\leq0.05$) overall acceptability, while redness was positively correlated ($r=0.93$, $p\leq0.05$) with overall acceptability of stuffed parothas during storage.

Table 1:- Proximate composition of stuffing prepared using green gram before and after germination

Parameters	**Green Gram (Ungerminated)**	**Green Gram (Germinated)**
Moisture	52.47±0.75	52.56±0.81
Protein	10.22±0.10	12.98±0.15
Fat	28.67±0.19	28.54±0.13
Total Ash	2.87±0.02	3.42±0.03
Crude Fibre	3.86±0.01	4.92±0.02
pH	6.72	6.64
aW	0.980	0.984
Vitamin C	4.01±0.02	7.82±0.01
B_1	0.08±0.01	0.12±0.01
B_2	0.11±0.1	0.16±0.3
Carotenoids	20.4±0.10	32.4±0.14

Table 2:- Standardisation of Stuffing and dough ratio for the preparation of stuffed parothas

Ratio (%)		Sensory scores		
Dough	Stuffing	Taste	Texture	OAA
50	50	7.0^{a}	7.0^{a}	7.0^{a}
55	45	7.5^{b}	7.4^{b}	7.5^{b}
60	40	8.5^{c}	8.5^{c}	8.5^{c}
65	35	8.2^{d}	8.0^{d}	8.1^{d}
70	30	8.0^{d}	7.8^{e}	7.9^{e}

*mean ± SD (n=15)

Table 3:- Proximate composition of stuffed parothas prepared using green gram before and after germination

Parameters	**Green Gram (Un germinated)**	**Green Gram (Germinated)**
Moisture	38.44±0.45	38.0±0.39

Protein	9.22±0.12	10.8±0.15
Fat	18.34±0.21	19.29±0.23
Total Ash	2.72±0.08	3.50±0.07
Crude Fibre	3.28±0.02	4.4±0.03
pH	6.68	6.48
aW	0.972	0.970
Vitamin C	1.78±0.01	3.62±0.02
B_1	0.04±0.001	0.06±0.001
B_2	0.05±0.001	0.08±0.002
Carotenoids	12.20±0.10	18.42±0.15

Table 4:- Chemical changes in stuffed parothas prepared using germinated green gram stored at ambient temperature conditions

Storage period (months)	Moisture* (%)	PV* (meqO_2/Kg fat)	FFA (% Oleic acid)*	TBA (mgMA/Kg sample)*	Browning*	Sorbic acid*	OAA**
0	38.44^{a}	5.12^{a}	0.75^{a}	0.087^{a}	0.082^{a}	0.148^{a}	8.5^{a}
3	38.10^{a}	9.62^{b}	0.92^{b}	0.092^{b}	0.089^{b}	0.147^{a}	8.4^{a}
6	37.89^{bx}	15.72^{c}	1.21^{c}	0.102^{c}	0.098^{c}	0.144^{b}	7.9^{b}
9	37.52^{bx}	19.24^{d}	1.69^{d}	0.142^{d}	0.112^{d}	0.141^{b}	7.5^{c}

*mean ±SD (n=3)
**mean±SD (n=15)

Table 5:- changes in vitamin contents (B_1 & B_2), vitamin C and carotenoids in parothas prepared using germinated green graam during storage stored at ambient temperature conditions

Storage period (months)	Vitamin B_1 (mg/100g)	Vitamin B_2 (mg/100g)	Vitamin C (mg/100g)	Carotenoids (µg/g)
0	0.060^{a}	0.08^{a}	3.62^{a}	18.42^{a}
3	0.051^{b}	0.071^{b}	3.01^{b}	16.30^{b}
6	0.042^{c}	0.058^{c}	2.42^{c}	13.49^{c}
9	0.031^{d}	0.041^{d}	2.12^{d}	12.04^{d}

*mean ±SD (n=3)

Table 6:- changes in colour values in parothas prepared using germinated green gram during storage stored at ambient temperature conditions

Storage period (months)	L	a	b
0	50.45^{a}	5.10^{a}	20.45^{a}
3	48.24^{b}	6.13^{b}	18.42^{b}
6	45.16^{c}	8.01^{c}	15.12^{c}
9	40.32^{d}	10.36^{d}	11.20^{d}

*mmean ±SD (n=3)

Table 7:- changes in Textural attributes in parothas prepared using germinated green gram during storage stored at ambient temperature conditions

Storage period (months)	Hardness (N)	Springiness (mm)	Chewiness (N,mm)
0	3.90^{a}	0.71^{a}	0.61^{a}
3	4.55^{b}	0.65^{b}	0.70^{b}
6	6.32^{c}	0.58^{c}	0.91^{c}
9	9.65^{d}	0.49^{d}	1.20^{d}

*mean ±SD (n=3)

Table 8:- changes in microbiological profile of parothas prepared using germinated green gram during storage stored at ambient temperature conditions

Storage period (months)	SPC	Coliform	Y&M
0	Nil	Nil	Nil
3	Nil	Nil	Nil
6	Nil	Nil	Nil
9	Nil	Nil	Nil

Conclusions:-

This study discovers the possible synergistic effect of antimycotic agent and inpack heat treatment on the extension of shelf life of stuffed parothas prepared by using germinated green gram. Germinated green gram as a good source of protein, fibre and vitamins seems to be suitable for the preparation of nutritious stuffed parothas with longer shelf life having better sensory attributes. These parothas also can be used without using any adjuncts for consumption.

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